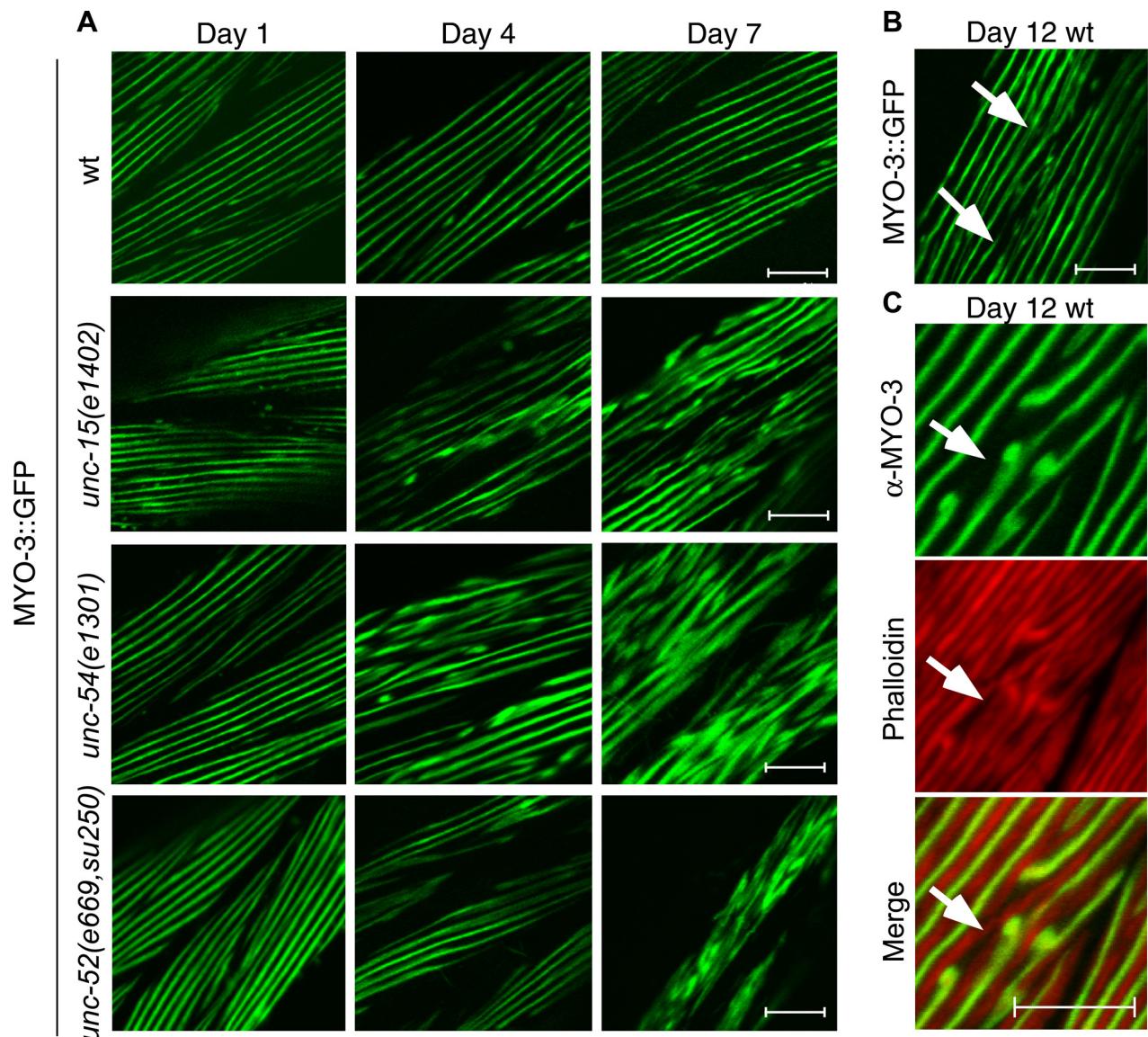
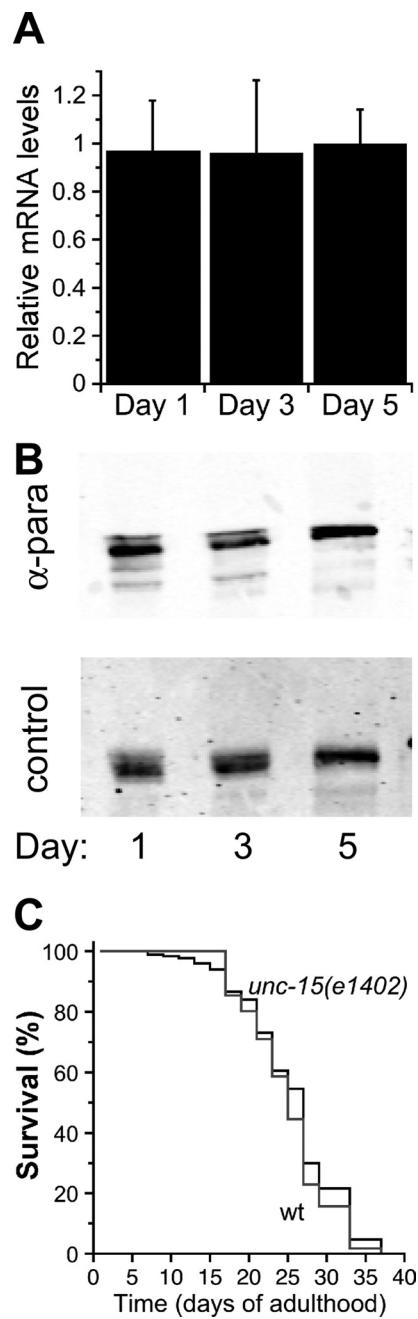


# Supporting Information

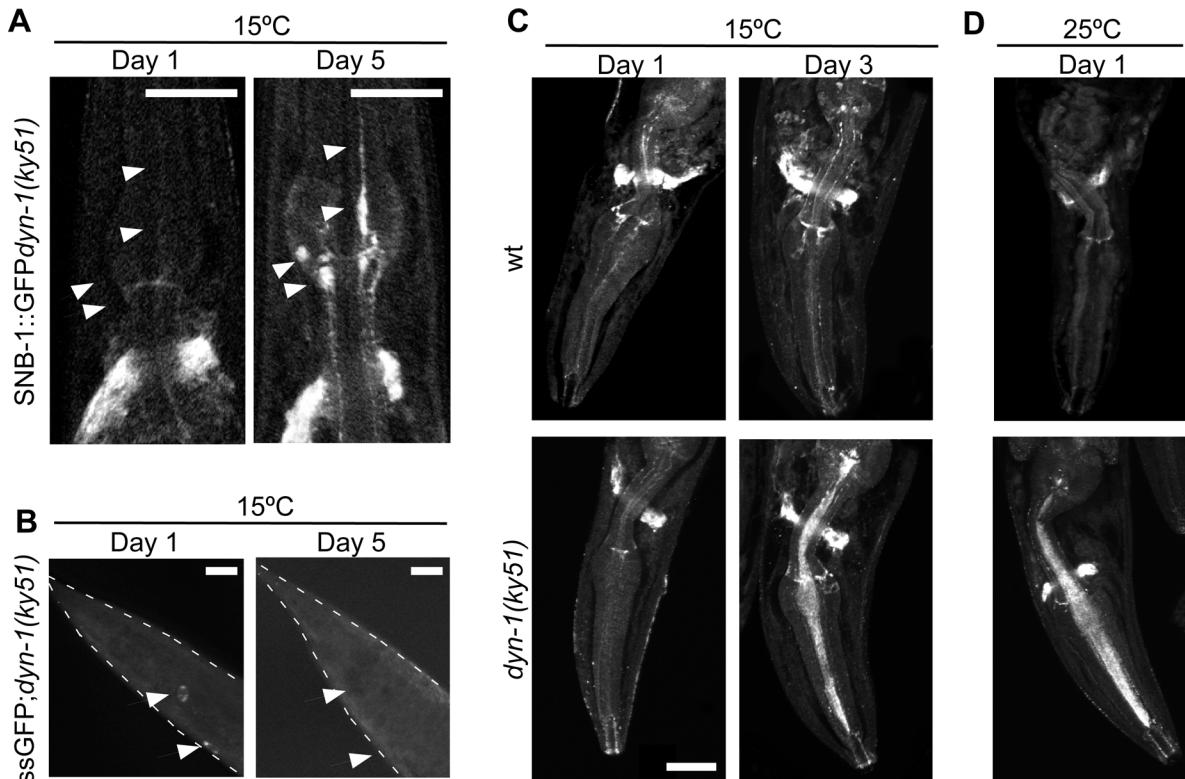
Ben-Zvi et al. 10.1073/pnas.0902882106



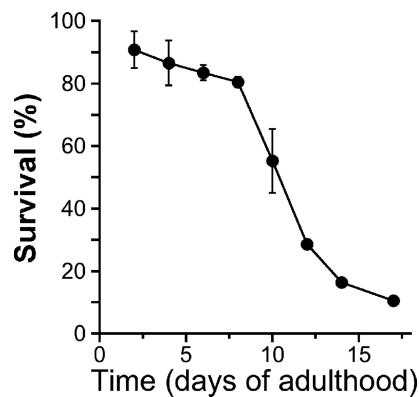
**Fig. S1.** Age-associated muscle deterioration is dependent on the specific ts protein expressed. Confocal images of age-synchronized animals grown at 15 °C. (A and B) MYO-3::GFP fluorescence of animals expressing WT or ts paramyosin (*unc-15*), myosin (*unc-54*), and perlecan (*unc-52*). (C) Day 12 WT animals stained with anti-myosin heavy chain A ( $\alpha\text{-MYO-3}$ ) and phalloidin. Arrows denote sarcopenia. (Scale bar: 10  $\mu\text{m}$ .)



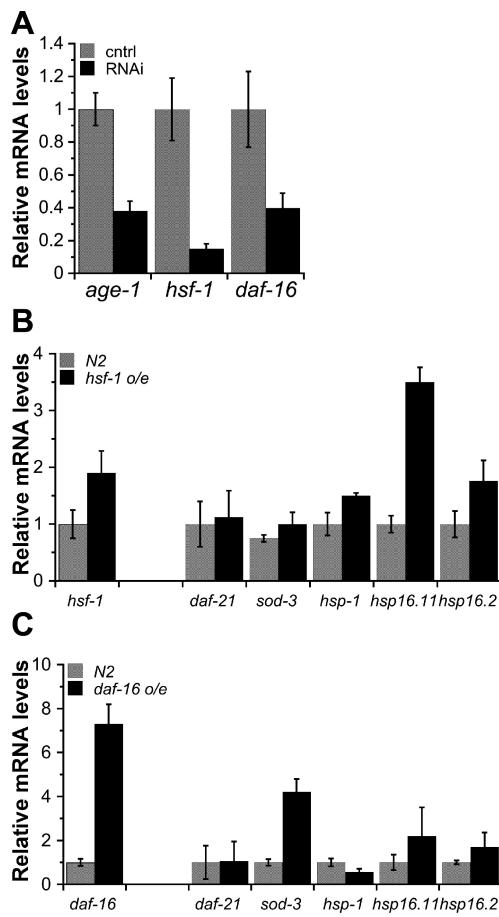
**Fig. S2.** Paramyosin ts-mutation does not affect expression levels or survival. (A) Time course of total paramyosin (UNC-15) mRNA levels of animals expressing paramyosin(ts). mRNA amounts were measured by quantitative RT-PCR and normalized to WT values. Data are mean  $\pm$  SE,  $>3$  independent biological samples. (B) Time course of total paramyosin protein levels of animals expressing paramyosin(ts). Protein extracts of age-synchronized animals were normalized to total protein and probed with paramyosin (Top) or dynamin (Bottom) antibodies. Images show a representative experiment. (C) Survival of animals expressing WT (gray) or ts (black) paramyosin ( $n > 200$  animals).



**Fig. S3.** The localization of dynamin(ts) and its substrates changes with age. (A and B) Representative confocal images of (A) SNB-1::GFP mislocalization or (B) ssGFP defective uptake scored in Fig. 2. Age-synchronized animals expressing dynamin(ts) and (A) SNB-1::GFP or (B) ssGFP were grown at 15 °C and imaged at day 1 and day 5 of adulthood. Arrows indicate age-dependent differences in (A) SNB-1::GFP and (B) ssGFP localization. (C and D) Confocal images of age-synchronized animals expressing WT or ts dynamin (*dyn-1*), grown at (C) 15 °C or (D) 25 °C and stained with anti-dynamin antibodies. (Scale bar: 10  $\mu$ m.)



**Fig. S4.** Animals' survival after heat shock decline with age. Survival of WT age-synchronized animals after extreme HS ( $35\text{ }^{\circ}\text{C}$ , 6 h). Data are mean  $\pm$  SD,  $>65$  animals per data point.



**Fig. S5.** Overexpression of DAF-16 or HSF-1 affects the expression levels of some downstream targets. (A) Total mRNA levels of control (gray) and *age-1*, *hsf-1*, or *daf-16* RNAi-treated animals (black) were measured by quantitative RT-PCR and normalized to control values. (B and C) Total mRNA levels of age-synchronized WT (gray), (B) HSF-1 (AM583), or (C) DAF-16 (TJ356) overexpressing (*o/e*) animals (black). Relative mRNA levels for *hsf-1*, *daf-16*, *daf-21*, *sod-3*, *hsp-1*, *hsp16.11*, or *hsp16.2* were measured by quantitative RT-PCR and normalized to WT values. Data are mean  $\pm$  SE,  $>3$  independent biological samples.

**Table S1. List of strains and abbreviations used in this work**

| Strain (ref) | Abbreviation               | Protein | Genotype   |
|--------------|----------------------------|---------|--|
| CB1402 (1)   | Paramyosin(ts)             | UNC-15  | unc-15(e1402)  |
| CB1301 (2)   | Myosin(ts)                 | UNC-54  | unc-54(e1301)  |
| CX51 (3)     | Dynamin(ts)                | DYN-1   | dyn-1(ky51)  |
| SD551 (4)    | ras(ts)                    | LET-60  | let-60(ga89)   |
| CW152 (5)    | gas-1(ts)                  | GAS-1   | gas-1(fc21)  |
| ZZ26 (6)     | Acetylcholine receptor(ts) | UNC-63  | unc-63(x26)  |
| HE250 (7)    | perlecan(ts)               | UNC-52  | unc-52(e669, su250)  |
| RW1596 (8)   | MYO-3::GFP                 | MYO-3   | myo-3(st386);stEx30[pmyo-3::myo-3::GFP;pRF4(rol-6(su1006))]      |
| NM306 (9)    | SNB-1::GFP                 | SNB-1   | j5ls1[psnb-1::snb-1::GFP;pRF4(rol-6(su1006))]                    |
| GS1919 (10)  | ssGFP                      | GFP     | dpy-20(e1282);arls37[pmyo-3::ssGFP;pMH86::dpy-20(+)]             |
| TJ356 (11)   | daf-16 o/e                 | DAF-16  | daf-16(+);zls356[pdaf-16::daf-16::GFP;pRF4(rol-6(su1006))]       |
| CF1407 (12)  | daf-16 o/e*                | DAF-16  | daf-16(mu86);muls71[pKL99::daf-16a::GFP/bKO;pRF4(rol-6(su1006))] |
| AM583 (13)   | hsf-1 o/e                  | HSF-1   | hsf-1(+);rmls249[plet-858::hsf-1;pmyo-2::gfp]                    |
| GF7 (14)     | hsf-1 o/e*                 | HSF-1   | hsf-1(+);dgEx7[plet-858::hsf-1;pRF4(rol-6(su1006))]              |

\*Different transgenic strains were used.

1. Gengyo-Ando K, Kagawa H (1991) Single charge change on the helical surface of the paramyosin rod dramatically disrupts thick filament assembly in *Caenorhabditis elegans*. *J Mol Biol* 219(3):429–441.
2. Mackenzie JM, Jr, Schachat F, Epstein HF (1978) Immunocytochemical localization of two myosins within the same muscle cells in *Caenorhabditis elegans*. *Cell* 15(2):413–419.
3. Clark SG, Shurland DL, Meyerowitz EM, Bargmann CI, van der Bliek AM (1997) A dynamin GTPase mutation causes a rapid and reversible temperature-inducible locomotion defect in *C. elegans*. *Proc Natl Acad Sci USA* 94(19):10438–10443.
4. Eisenmann DM, Kim SK (1997) Mechanism of activation of the *Caenorhabditis elegans* ras homologue let-60 by a novel, temperature-sensitive, gain-of-function mutation. *Genetics* 146(2):553–565.
5. Kayser EB, Hoppel CL, Morgan PG, Sedensky MM (2003) A mutation in mitochondrial complex I increases ethanol sensitivity in *Caenorhabditis elegans*. *Alcohol Clin Exp Res* 27(4):584–592.
6. Lewis JA, Wu CH, Berg H, Levine JH (1980) The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. *Genetics* 95(4):905–928.
7. Mackenzie JM, Jr, Garcea RL, Zengel JM, Epstein HF (1978) Muscle development in *Caenorhabditis elegans*: Mutants exhibiting retarded sarcomere construction. *Cell* 15(3):751–762.
8. Campagnola PJ, et al. (2002) Three-dimensional high-resolution second-harmonic generation imaging of endogenous structural proteins in biological tissues. *Biophys J* 82(1 Pt 1):493–508.
9. Nonet ML (1999) Visualization of synaptic specializations in live *C. elegans* with synaptic vesicle protein-GFP fusions. *J Neurosci Methods* 89(1):33–40.
10. Fares H, Greenwald I (2001) Genetic analysis of endocytosis in *Caenorhabditis elegans*: Coelomocyte uptake defective mutants. *Genetics* 159(1):133–145.
11. Henderson ST, Johnson TE (2001) daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr Biol* 11(24):1975–1980.
12. Lin K, Hsin H, Libina N, Kenyon C (2001) Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet* 28(2):139–145.
13. Morley JF, Morimoto RI (2004) Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol Biol Cell* 15(2):657–664.
14. Mohri-Shioiri A, Garsin DA (2008) Insulin signaling and the heat shock response modulate protein homeostasis in the *Caenorhabditis elegans* intestine during infection. *J Biol Chem* 283(1):194–201.

**Table S2. List of crosses used in this work**

| Strain | Genotype  |
|--------|---|
| AM703  | unc-15(e1402);myo-3(st386);stEx30[pmyo-3::myo-3::GFP;pRF4(rol-6(su1006))]           |
| AM704  | unc-54(e1301); myo-3(st386);stEx30[pmyo-3::myo-3::GFP;pRF4(rol-6(su1006))]          |
| AM705  | unc-52(e669, su250);<br>myo-3(st386);stEx30[pmyo-3::myo-3::GFP;pRF4(rol-6(su1006))] |
| AM706  | dyn-1(ky51); jsIs1[psnb-1::snb-1::GFP;pRF4(rol-6(su1006))]                          |
| AM528  | dyn-1(ky51); arls37[pmyo-3::ssGFP;pMH86::dpy-20(+)]                                 |
| AM707  | dyn-1(ky51);daf-16(+);zIs356[pdaf-16::daf-16::GFP;pRF4(rol-6(su1006))]              |
| AM586  | dyn-1(ky51);hsf-1(+);rmls249[plet-858::hsf-1;pmyo-2::gfp]                           |
| AM558  | let-60(ga89);daf-16(+);muls71[pKL99::daf-16a::GFP/bKO;pRF4(rol-6(su1006))]          |
| AM708  | let-60(ga89);hsf-1(+);dgEx7[plet-858::hsf-1;pRF4(rol-6(su1006))]                    |

**Table S3. List of primer sequences**

| Gene                    | Sequence   |
|-------------------------|--|
| <i>Actin</i><br>(Act-4) | Forward: 5'-ATC ACC GCT CTT GCC CCA TC-3'<br>Reverse: 5'-GGC CGG ACT CGT CGT ATT CTT G-3'            |
| <i>daf-16</i>           | Forward: 5'-GTG CCA AGC ACT AAC TTC AAG CC-3'<br>Reverse: 5'-CCA CAC GAT TGA ATT CCA GGC AGT G-3'    |
| <i>hsf-1</i>            | Forward: 5'-GCA TAA CAA TAT GAA TAG CAT GGT C-3'<br>Reverse: 5'-GAC GTC CTT GTA CAA AAC ACG GAT G-3' |
| <i>daf-21</i>           | Forward: 5'-CGC TAC CAG GCA CTC ACC GAG-3'<br>Reverse: 5'-GGA CAA GCT CTT GTA GAA CTC AG-3'          |
| <i>sod-3</i>            | Forward: 5'-GCT GCA ATC TAC TGC TCG CAC TG-3'<br>Reverse: 5'- GGC TGA TTA CAG GTT CCA AAT CTG C-3'   |
| <i>hsp-1</i>            | Forward: 5'-CTC GAG TCA TAC GCC TTC AAC CTT A-3'<br>Reverse: 5'-GGC CAA TCC TTC CAA ATC CTT CTG-3'   |
| <i>hsp-16.11</i>        | Forward: 5'-GTG ATC TCA TGA GAG ATA TGG-3'<br>Reverse: 5'-CAA CGG GCG CTT GCT GAA TTG G-3'           |
| <i>hsp-16.2</i>         | Forward: 5'-ACT TTA CCA CTA TTT CCG TCC AGC-3'<br>Reverse: 5'-CCT TGA ACC GCT TCT TTC TTT G-3'       |
| <i>unc-15</i>           | Forward: 5'-GGA GGA TAC TCA ACG TCA GTT GC-3'<br>Reverse: 5'-CGG ATA GCG TTG TCG AGA GCG G-3'        |
| <i>18S rRNA</i>         | Forward: 5'-GCC AGC AGC CGC GGT AAT TCC AGC-3'<br>Reverse: 5'-TTG CGA ATC TGA GGC ACG TAA CCT-3'     |
| <i>C12C8.1</i>          | Forward: 5'-ACT CAT GTG TCG GTA TTT ATC-3'<br>Reverse: 5'-ACG GGC TTT CCT TGT TTT G-3'               |
| <i>F44E5.4</i>          | Forward: 5'-AAT GAA CCA ACT GCTGCT CTT-3'<br>Reverse: 5'-CCT TTC CGG TCT TCC TTT TG-3'               |
| <i>hsp-4</i>            | Forward: 5'-GTG GCA AAC GCG TAC TGT GAT GA-3'<br>Reverse: 5'-CGC AAC GTA TGA TGG AGT GAT TCT-3'      |
| <i>mtl-1</i>            | Forward: 5'-GGC TTG CAA GTG TGA CTG CAA AAA C-3'<br>Reverse: 5'-CTT GCA GTC TCC CTT ACA TCC AGC-3'   |